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THE EFFECT OF PTERIN PIGMENTS ON WING COLORATION OF FOUR SPECIES OF PIERIDAE (LEPIDOPTERA)

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THE PTERINS ARE A CLASS of chemical compounds with a widespread but sporadic distribution throughout the animal kingdom. They were first isolated from pierid butterflies where they are responsible for the orange, yellow and white coloration of the wings. Although the Pieridae are the only butterflies from which pterins have been reported, these pigments have been identified in representatives of other insect orders and in crustaceans. Pterins have also been reported from certain fish, amphibians, reptiles and mammals (Fox and Vevers, 1960).

The first major study of pterins was undertaken by F. Gowland Hopkins (1895), who extracted these white, yellow and red pigments from various Pieridae. At the time, he did not realize he was working with an entirely new class of chemical compounds. In fact, he identified the white pigment as uric acid and stated that the yellow and red pigments were close relatives of uric acid. By means of more refined chemical tests, it is now known that the pigments Hopkins extracted were indeed pterins.

The object of this study was to extract and identify the wing pterins of several species of pierids and to determine if there was any correlation between wing color and pterin content. The four species finally chosen for intensive study included the white *Pieris rapae* (Linnaeus), the yellow *Colias harfordii* H. Edwards, the yellow-orange *Colias eurytheme* Boisduval, and the orange *Eurema nicippe* (Cramer).

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Table I

R_F values for pterins.

	Xantho	Isoxantho	Erythro	Leuco	Sepia
P. rapae					
Propanol	.14	.14	.06	.06	.39
Butanol	.35	.22	.10	.08	.35
C. harfordii					
Propanol	.14	.14	.03	.05	.35
Butanol	.34	.23	.09	.08	.34
C. eurytheme					
Propanol	.13	.13	.03	.05	.35
Butanol	.35	.22	.09	.08	.35
E. nicippe					
Propanol	.13	.13	.03	.05	.35
Butanol	.32	.22	.08	.06	.32
Standards					
Propanol	.15	.17	.05	.07	---
Butanol	.30	.19	.08	.07	---

The pterin pigments from the four wings of a dried specimen were extracted with 2 ml of 1% ammonia after washing the wings in about 10 ml of acetone. The pigments were then applied to Whatman No. 1 chromatography paper, the spots being about 2-3 mm in diameter. The chromatograms were developed by means of descending paper chromatography using 7:3 propanol/1% ammonia and 4:1:1 butanol/acetic acid/water as solvents. The solvent front was allowed to advance about 15 cm on the paper. The chromatograms were then removed from the chromatography tank and dried with a hair drier. The pterin spots were located under long-wave ultraviolet light and identified by their characteristic fluorescing colors and Rf values.

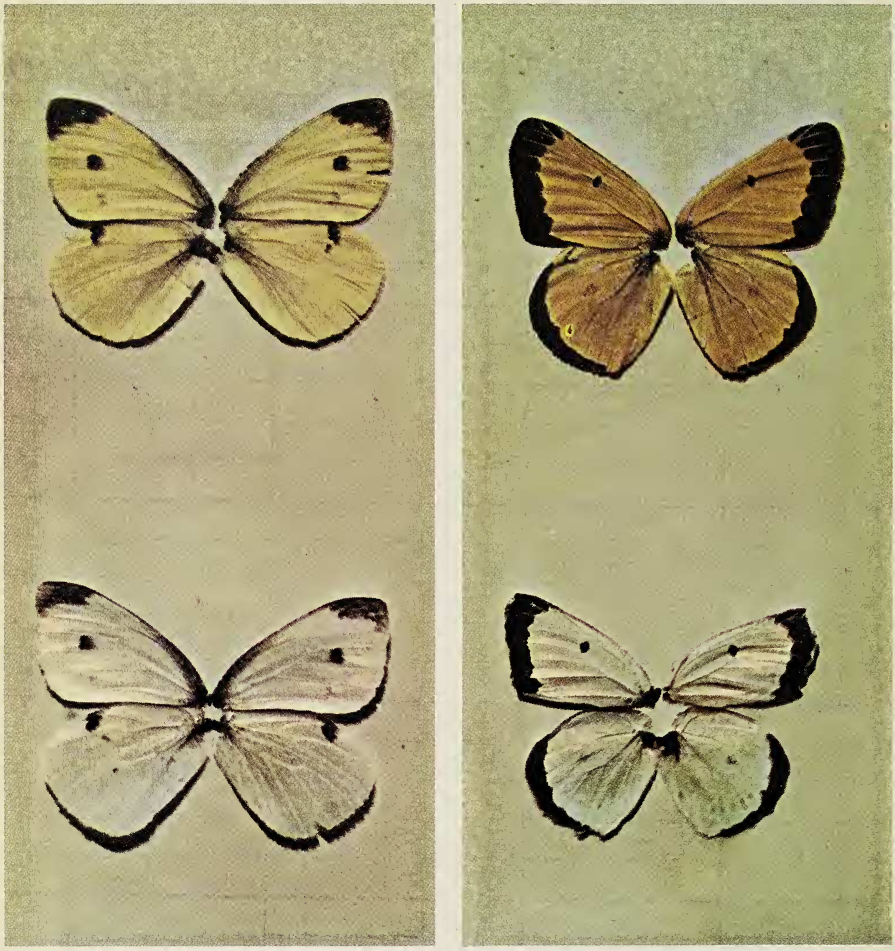
Standards were established by chromatographing known pterins (isoxanthopterin and erythropterin from Aldrich Chemical Co.; xanthopterin and leucopterin from K and K Laboratories). A small quantity of each standard was dissolved in a few ml of 1% ammonia and run separately, according to the procedures outlined above.

RESULTS

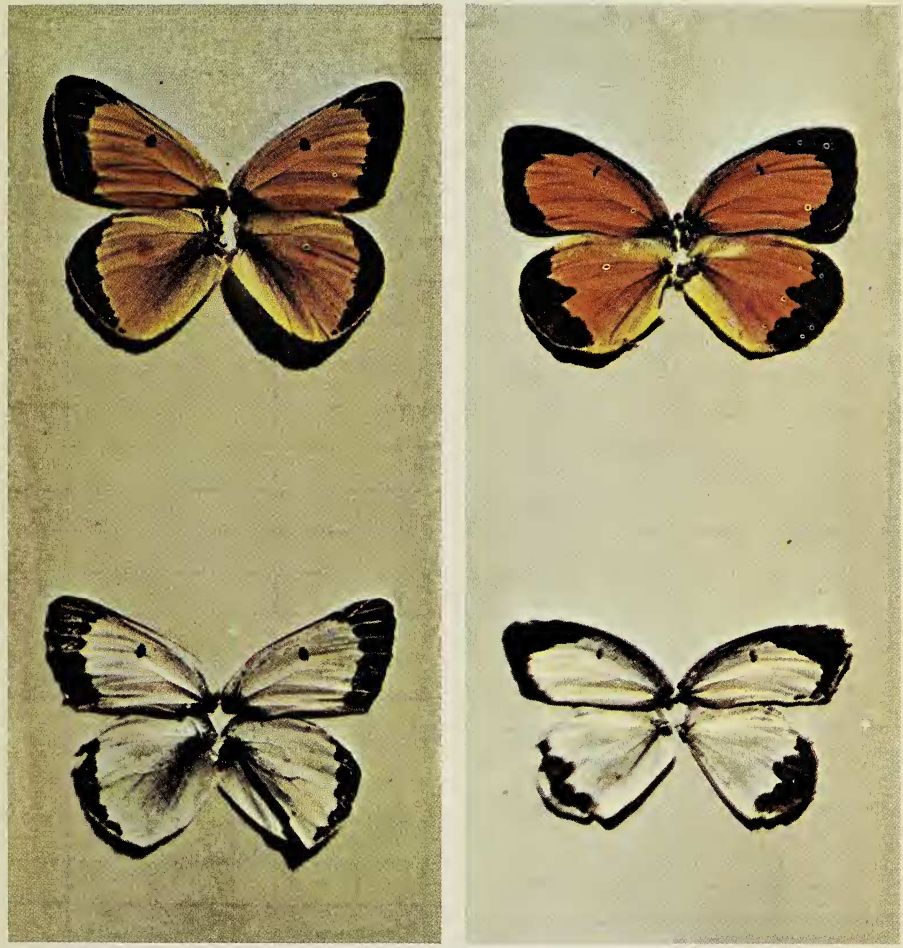
In Table I the average Rf values obtained for the pigments of the four species of Pieridae are compared with those obtained for the standards. The values represent averages compiled from the results of a large number of chromatograms. Table II compares the UV fluorescing color with the actual color of the pigments. Using both Rf values and fluorescing colors, it was found that each species of Pieridae contained the same five pigments.

DISCUSSION

Watt (1964) also characterized the pterin pigments of *Colias eurytheme*. Table III compares my results with those of Watt and in addition shows the Rf values for the standards. All determinations were made using 4:1:1 butanol/acetic acid/water and Whatman No. 1 paper. I was unable to obtain a standard of sepiapterin, but the Rf value and color of fluorescence observed for this unidentified spot agree very closely with the literature for that of sepiapterin (Harmsen, 1966; Watt, 1964).



Figures 1-4. 1. *Pieris rapae,* 2. *Colias harfordii,* 3. *Colias eurytheme,* 4. *Eurema nicippe.* The upper specimen (minus the body) in each figure shows the typical phenotypic coloration of the species. The lower specimen shows the effect of removing the wing pterins. In each case the lower specimen has a pearly lustre which is due entirely to a structural effect. When the



pterins are removed the scales are not affected and the reflected light gives the white appearance. The dark color that is found on the wings as spots and/or marginal bands is due to the presence of melanin. The melanin is insoluble in 1% ammonia and therefore is not extracted with the pterins.

Table II

Color of pterins.

Pigment	UV	Visible
Xanthopterin	Yellow-Green	Yellow
Erythropterin	Reddish-Orange	Red
Isoxanthopterin	Bright Blue	White
Leucopterin	Pale Blue	White
Sepiapterin	Yellow	Yellow

Table III

R_F values for pigments of C. eurytheme and standards.

	Xantho	Isoxantho	Erythro	Leuco	Sepia
Standard	.30	.19	.08	.07	---
1	.33	.21	.11	.07	.33
2	.35	.22	.09	.08	.35

All four species of Pieridae were shown to contain the same five pterin pigments but in varying amounts. By assuming a correlation between strength of fluorescence and amount of pigment present, the relative quantities of each pigment could be estimated. *P. rapae* contained large amounts of isoxanthopterin and leucopterin and smaller amounts of the other three pigments. *C. harfordii, C. eurytheme* and *E. nicippe* contained lesser amounts of the white pigments and large amounts of the yellow pigments, especially xanthopterin. The largest amounts of erythropterin were detected in *E. nicippe.*

It can then be concluded that in this representative sample of pierids, the whites, yellows, and oranges of the wings are due to the differential concentration of certain pterins.

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HABITAT — *Zerene caesonia eurydice* Bdv.

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Amorpha californica occurs from sea level to 8000 feet in elevation. The habitat shown (Fig. 1) is at 6500 feet in the Mill Creek Canyon, San Bernardino Mountains, California, taken May 31, 1970. Here *Zerene* is present in a highly variable population, mostly in the forms known as *eurydice* but with a continuous range of variability to *caesonia*. *Amorpha* are the bushes, ranging from one to four feet high, shown around the rocks (Fig. 1). A plant is shown in closer view in Fig. 2.

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